Expression of inflammatory mediators in periodontitis and T2D patients: a systematic review and meta-analysis

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Abstract: The high concentration of glucose in the blood in Type 2 diabetes (T2D) may be related to either insulin resistance or insulin deficiency. Moreover, the literature points to periodontitis as the main oral disease caused by glycemia imbalance. The quantification of inflammatory markers in blood or saliva samples of T2D patients may represent a valuable tool in revealing how well an individual's immune system can respond to injuries and periodontal treatment. In addition, an evaluation of the cytokine expression is extremely relevant to help understand the connection between periodontitis and T2D. This systematic review and meta-analysis aimed to evaluate the expression of inflammatory markers in T2D patients with periodontitis, compared with non-diabetic patients with periodontitis. A total of 3,894 studies were retrieved after a systematic literature search, 15 of which were included in the systematic review, and 4 of these 15, in the meta-analysis. The results did not indicate any statistical difference between the groups regarding TNF-α and IL-6 markers. T2D patients with periodontitis had increased levels of IL-10, compared with non-diabetic individuals with periodontitis ($p = 0.003$). On the other hand, the IL-4 concentration in non-diabetic individuals with periodontitis was high, compared with the T2D group ($p < 0.001$). Several studies did not include quantitative results and were excluded from the meta-analysis. The high IL-10 expression and low IL-4 expression in the T2D group suggest an association between the level of these markers and the impairment of the immune response in T2D patients with periodontitis.

Keywords: Periodontitis; Diabetes Mellitus, Type 2; Cytokines; Blood; Saliva.

Introduction

Periodontitis is a chronic multifactorial inflammatory disease that is associated with dysbiotic biofilms, and is characterized by progressive destruction of the tooth-supporting apparatus.[1] Periodontal infection with pathogenic microorganisms, mainly gram-negative anaerobes, is the primary etiological cause of periodontitis. This disease is associated with loss of periodontal ligament attachment and alveolar bone insertion, ultimately leading to the apical migration of the junctional epithelium, with the formation of periodontal pockets.[2] This outcome seems to

be exacerbated in type 2 diabetes (T2D) patients. Periodontitis represents the sixth major complication of diabetes.[3] Poor glycemic control leads to the worsening of periodontal conditions, and promotion of periodontal disease as a risk factor for diabetes.[4;5] Diabetes increases the prevalence and severity of gingivitis and periodontitis, which can be submitted to periodontal treatment to reduce glycated hemoglobin levels and gain metabolic control of T2D.[6]

The inflammatory response of the host plays a fundamental role, together with the bacterial etiology.[7] The presence of microorganisms in the oral cavity is not sufficient in itself to characterize them as pathobionts in T2D-related periodontitis. The imbalance of the immune and inflammatory response of the host may be associated with the establishment, progression, and tissue collapse of periodontal disease.[8] This disease induces different immune responses, according to individual susceptibility, and different degrees of clinical severity. Additionally, environmental, genetic, and behavioral factors are related to the development of the disease in susceptible individuals.[9] A broad range of cytokines and chemokines have been reported to be significantly greater in the gingival crevicular fluid of T2D individuals with periodontitis, compared with systemically healthy patients. The high expression of proinflammatory cytokine interleukin-1β, interleukin-6 and tumor necrosis factor-α (TNF-α), in both periodontal tissue and gingival crevicular fluid, may influence the local conditions and the immune reaction in periodontal tissues, ultimately predisposing DM patients to tissue damage.[10] Moreover, elevated blood-glucose levels lead to excessive accumulation of advanced glycation end-products (AGEs) in the serum, cells, and tissues. The interaction between AGEs and their receptor (RAGE) leads to cellular oxidative stress, resulting in excessive production of reactive oxygen species (ROS), and secretion of inflammatory cytokines, such as TNF-α and interleukin-1β. The association of these two factors results in both alveolar bone resorption and destruction of collagen fibers, because of the increased production of matrix metalloproteinases by macrophages and osteoclasts.[11,12] Different immune responses to periodontal disease can be expected among different individuals; therefore, there is no way to standardize a specific type of response. In T2D, the presence of inflammatory mediators may be indicative of the risk for further health disorders, and a marker for periodontal disease progression or severity.

The balance between pro- and anti-inflammatory cytokines is responsible for several processes involving the immune system and the immune response. Anti-inflammatory cytokines tend to attenuate the inflammatory process, while pro-inflammatory cytokines tend to exacerbate it. Therefore, by measuring the levels of these mediators in T2D patients with periodontitis, an overview can be made of the individual's immune system response, and certain issues can be elucidated, such as the inflammatory cascade in both diseases, its role, and its potential for tissue repair or destruction. However, the quantification of the levels of multiple inflammatory markers, and the correlation between their expression and the periodontal involvement have proven difficult to measure.[13] The clinical parameters of periodontitis and periodontal inflammation provide only qualitative data for evaluating the response.[13] A biomarker that could indicate both the periodontal condition and the stage of disease progression could guide the clinician's diagnosis and treatment protocols.[2] The discovery of such a biomarker to complement the clinical diagnosis could help clinicians understand the progression of the disease, and thus propose a treatment plan based on clinical and immunological aspects. A marker that could aggravate periodontal disease could also offer a more controlled and specific treatment. Although clinical parameters have thus far been sufficient for decision making, they cannot be applied to predict what course the disease will take, or whether it may develop into a more severe form at some point. This systematic review aims to evaluate quantitative and qualitative data on the expression of inflammatory mediators in the blood and saliva of T2D patients with periodontitis.

Question formulation

This systematic review aimed to evaluate the expression of pro- and anti-inflammatory markers in T2D patients with periodontitis, compared with non-

diabetic patients with periodontitis, by answering the following focused question: "What is the expression of pro- and anti-inflammatory markers involved in the process of bone loss and periodontal repair in the blood and saliva of T2D patients with periodontitis, compared with non-diabetics with periodontitis?"

Critical evaluation and data collection

Protocol and registration

This systematic review with meta-analysis was registered at the International Prospective Registries of Systematic Reviews (PROSPERO) on October 9, 2019, under protocol number CRD42020154010. The study followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist (PRISMA).[14]

Inclusion and exclusion criteria

The PECO strategy[15] was used to design the research toward understanding the expression of pro- and anti-inflammatory markers involved in the process of bone loss and periodontal repair in the blood and saliva of T2D patients with periodontitis. The strategy consisted of the following acronym representations: **P**atients with periodontitis; **E**xposure to T2D; **C**omparison with nondiabetic patients; and **O**utcome representing the concentration of pro- and anti-inflammatory markers in the blood and saliva. Observational cohort, cross-sectional, and case-control studies were included.

The studies that included patients who smoked or had any additional systemic conditions that could alter the markers were excluded from review. Also excluded were studies that included Type 1 diabetic patients; studies analyzing markers other than cytokines, chemokines, and interleukins (IL), or proteases, enzymes, oxidative stress factors, or lipid and genetic evaluations; clinical trials calling for interventions and/or offering therapies; and studies that performed analyses using a gingival biopsy or gingival crevicular fluid. Any biomarkers that might suggest a relationship between periodontal disease and Type 2 diabetes were researched, as shown in Table 1, and in the exclusion criteria. No language was excluded, and no time period was stipulated.

The search strategy was constructed according to the terms of Medical Subject Headings (MeSH), initially developed for PubMed, and later adapted for other databases. The surveys were conducted on March 17, 2020, and updated on August 13, 2021. All the terms, synonyms, and words with different spellings were used. PubMed, Cochrane, BVS (LILACS), Web of Science, Scopus, Livivo, ProQuest, OpenGrey, Google Scholar, and the gray literature were searched. Moreover, manual searches were made in the reference lists of the included studies, and experts were consulted. The search strategies for each database and gray literature can be accessed at https://osf.io/a53bc/files/ .

Details taken from the strategy used in each database are available upon request. The reference manager used for the organization and exclusion of duplicates was EndNote Web (Clarivate Analytics Web of Science Group). A list of the studies excluded, together with the reasons, can be accessed at https://osf.io/a53bc/files/.

Study selection

In the first phase of the study selection, two independent reviewers (MM, LG) evaluated the titles and abstracts of all the articles retrieved, based on the inclusion criteria. In the second phase, the same two reviewers evaluated the full texts based on the inclusion and exclusion criteria. A third reviewer (CMS) was not needed, because there were no conflicts to be solved. Studies with inaccessible full texts were excluded. Emails were sent to the authors requesting the complete text, but no author responded.

Data extraction

Data extraction was performed by two independent reviewers, and any conflicts emerging thereafter were solved by consensus. The extracted data comprised the authors, year of study, country, characteristics of the participants (mean age/standard deviation or range), periodontitis case-definition, T2D case definition by the standard level of glycated hemoglobin, means of biomarkers analysis (saliva or serum/plasma), pro/anti-inflammatory markers tested, and the outcomes and conclusions of each study.

Table 1. Descriptive characteristics of the included studies (n = 15).
P: Periodontitis group; DMP: Diabetes and Periodontitis group. ANP: Age not provided. IL= Interleukin; *No numerical data is available.

Author, year, country	AGE (Average and SD/ Amplitude)	n. of groups	Periodontitis case definition	Study design	HbA1C (Type 2 diabetes)	Outcome	Results
Acharya et al., 2015,[19] India	42.7/ 30–55	P: 15 DMP: 15	≥ 20 natural teeth; ≥ 4 teeth with probing depth ≥ 5mm; clinical attachment loss ≥ 2mm; bleeding on probing; radiographic bone loss (long cone technique).	Cross-sectional	≥ 7%	Serum; IL-10	P: 10.50±0.61 pg/ml DMP 11.35±0.97 pg/ml (p=0.374)
Acharya et al., 2016,[20] India	30–55	P: 20 DMP: 20	Probing depth ≥ 5mm; generalized bleeding; generalized clinical attachment loss ≥ 2mm; radiographic bone loss.	Cross-sectional	7.5%–9.5%	Serum; TNF-α, IL-4, IL-6	* **IL-4:** Increased in group P (P=0.172). **TNF-α** increased in group DMP compared with group P (P <0.05); IL-6 increased in group DMP compared with group P (p<0.05).
Acharya et al., 2017,[21] India	30–55	P: 20 DMP: 20	Individuals diagnosed with chronic periodontitis (moderate to severe).	Cross-sectional	7.5%–9.5%	Serum; TNF-α; IL-4, IL-6, IL-1β, IL-10	**TNF-α**: Groups P/DMP: 16.66 pg/ml/19.78pg/ml **IL-4:** Groups P/DMP: 22.30 pg/ml/20.20 pg/ml **IL-6:** Groups P/DMP: 19.99 pg/ml/30.25 pg/ml **IL-1β:** Groups P/DMP: 4.63 pg/ml/6.16 pg/ml **IL-10:** Groups P/DMP: 10.46 pg/ml/11.36 pg/ml Median values. The study showed no comparison between the p group and the DMP group. It presented a comparison between 4 groups.
Bakshi et al., 2018,[24] India	51 ± 8.2	P: 15 DMP: 15	Probing depth ≥ 5mm; generalized clinical attachment loss ≥ 2mm; radiographic bone loss.	Cross-sectional	P: 5.2 ± 0.30 DMP: 7.8 ± 0.40	Plasma; TNF-α, IL-4,IL-6	**TNF-α** Groups P/DMP: 15.8±0.40/ 27.3± 0.32; **IL-6** Groups P/DMP: 22.02±0.34/39.8±0.35; **IL-4** Groups P/DMP: 30.5±0.20/ 25.05±0.40 Note: No unit of measurement quoted.

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Balaji et al., 2017,[22] India	35–65	P: 20 DMP: 20	≥ 15 natural teeth (excluding third molars); presence of interproximal loss ≥ 3mm in two or more non adjacent teeth.	Cross-sectional	6%	Saliva; IL-6	Group DMP: 245.42±46.81. Group P: 123.39 ± 50.47. Note: No unit of measurement quoted.
Balitska, 2019,[29] Ukraine	ANP	P: 32 DMP: 32	Diagnosis of periodontal disease confirmed by anamnesis, clinical examination, determination of hygiene and periodontal indexes; radiographs.	Cross-sectional	-	Serum; IL-4, IL-10	**IL-4** Groups P/DMP: 2.98/1.48 pg/ml (median) **IL-10** Groups P/DMP: 6.79/3.39 pg/ml (median)
Costa et al., 2010,[27] Brazil	P: 45.4 ± 1.2 (SE) DMP: 47.8 ± 1.4 (SE)	P: 24 DMP: 24	≥ 15 natural teeth (excluding third molars); proximal insertion loss ≥ 3mm in two or more non adjacent teeth.	Cross-sectional	6%	Saliva; IL-6	*IL-6 levels were higher in groups P and DMP (p = 0.006). IL-6 was positively correlated with HbA1c levels in the periodontitis diabetes group (p = 0.04).
Guruprasad et al., 2018,[23] India	P: 41.5 ± 6.56 DMP: 48.5 ± 6.55	P: 35 DMP: 35	Gingival inflammation; gingival index > 1; probing depth ≥ 4mm; clinical attachment loss ≥ 1mm; evidence of radiographic bone loss.	Cross-sectional	6.5%–7%	Plasma;IL-34	P: 608.17 ± 167.38 pg/ml DMP: 671.84 ± 212.14 pg/mg
Longo et al., 2014,[26] Brazil	P: 47.0 ± 5.25 DMP (adequate glycemic control): 60.6 ± 10.67 DMP (inadequate glycemic control): 52.7 ± 5.54	P: 06 DMP (adequate glycemic control): 10 DMP (inadequate glycemic control): 10	≥ 30% of the sites with probing depth > 4mm and bleeding on probing; ≥ 15 natural teeth.	Cross-sectional	adequate glycemic control: 6.83 ± 0,78; inadequate glycemic control: 10.86 ± 2.21 Non diabetics: 5.43 ± 0.54	Serum; IL-6, IL-8	* **IL-6** (p= 0.6351); **IL-8** (p=0.9460)
Maboudi et al., 2019,[30] Iran	P: 44.78 ± 13.18 DMP: 52.72 ± 9,96	P: 18 DMP: 18	Probing depth ≥ 3mm and clinical attachment loss ≥ 2mm.	Cross-sectional	≥ 6.5%	Serum; IL-23,IL-35	*The IL-23 and IL-35 interleukins did not present a significant statistical difference between the groups.

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Miranda et al., 2019,[28] Brazil	P: 52.7 ± 8.3 DMP: 55.9 ± 9.2	P: 26 DMP: 30	≥ 15 teeth (excluding third molars); > 30% of sites with probing depth and clinical attachment loss ≥ 4mm and bleeding on probing; and at least 6 teeth distributed in 4 quadrants with at least 1 site with probing depth and clinical attachment loss ≥ 5mm and bleeding on probing.	Cross-sectional	> 6.5%	Serum; IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17 A, IL-21, IL-23, TGF-β, IFN-γ, TNF-α	**IL-10** Groups P/DMP: 10±5.6/ 10.5±17 pg/ml **IL-4** Groups P/DMP: 21.8±14.9/ 16±21.6 pg/ml **IL-5** Groups P/DMP: 1.2±1.5/ 1.4±3.9 pg/ml **IL-13** Groups P/DMP: 10.4±20.6/ 11.7±46.4 pg/ml **IL-2** Groups P/DMP: 1.1±0.8/ 0.7±0.9 pg/ml ($p < 0.05$) **TGF-β** Groups P/DMP: 2.6±1.8/ 4.5±5.0 pg/ml **IL-8** Groups P/DMP: 5.1±5.3/ 6.8±9.8 pg/ml **IL-1β** Groups P/DMP: 1.1±0.8/ 0.7±1.1 pg/ml **TNF-α** Groups P/DMP: 4.7±2.9/ 4.4±2.4 pg/ml **IL-6** Groups P/DMP: 2.9±2.1/ 2.0±2.5 pg/ml **IFN-γ** Groups P/DMP: 5.8±2.4/ 3.8 ±3.8 pg/ml ($p < 0.05$) **IL-12** Groups P/DMP: 3.1±2.1/ 1.4±2.0 pg/ml ($p < 0.05$) **IL-17** Groups P/DMP: 8.4±3.4/ 7.1±7.0 pg/ml **IL-21** Groups P/DMP: 0.6±1.2/ 0.7±1.6 pg/ml **IL-23** Groups P/DMP: 258.2 ±218.2/ 255.7 ± 709.1 pg/ml **IL-7** Groups P/DMP: 6.8±4.1/ 5.2±4.7 pg/ml
Purnamasari et al., 2019,[31] Indonesia	P: 50.81 ± 12.50 DMP: 49.5 ± 8.78	P: 16 DMP: 22	Group P not severe: clinical attachment loss 1–4mm; Group P severe: clinical attachment loss > 5mm. Plaque index, probing depth and bleeding on probing were also evaluated.	Cross –Sectional	-	Serum; IL-10, TNF-α	**IL-10** Groups P/DMP (mean/standard deviation): 3.38±1.05/ 6.98±12.63 (P=0.079) **TNF-α** P/DMP groups (medium/range): 6.52(5.13-8.10)/ 6.16(5.43-12.94) (p=0.722) Note: No unit of measurement quoted.

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Saxena et al., 2020,[25] Índia	P: 50.4 ± 8.6	P: 17	Patients with sites presenting with CALs ≥ 4mm, PDs of ≥ 5mm, with ≥ 4 teeth in each jaw and ≥ 50% alveolar bone loss in ≥ 2 quadrants were defined as having CP.	Cross –Sectional	DMP (adequate glycemic control): ≥ 6.5 and < 7%	Saliva; IL-17	P: 56.54±19.79
	DMP (adequate glycemic control): 53.6 ± 9.0	DMP (adequate glycemic control): 17			DMP (inadequate glycemic control): ≥ 7%		DMP (adequate glycemic control): 63.84±24.72
	DMP (inadequate glycemic control):54.3 ± 8.8	DMP (inadequate glycemic control): 17					DMP (inadequate glycemic control): 74.74±17.79
							The study showed no comparison between the p group and the DMP group. It presented a comparison between 5 groups.
Singh et al., 2014,[2] India	P: 42.80 ± 8.02 (30-55) DMP: 45.10 ± 6.79 (32-53)	P: 20 DMP: 20	≥ 20 natural teeth; probing depth ≥ 5mm; clinical attachment loss ≥2mm.	Cross –Sectional	> 6%	Saliva; TNF-α	P: 8.46±4.60 ng/ml DMP: 26.52±8.52 ng/ml (p<0.001)
Techatanawat et al., 2020,[32], Thailand	P: 55.5 (50.25–60.50) DMP: 63 (55.0–69.0) (Values in median and interquartile range- 1st and 3rd)	P: 8 DMP: 23	PSR evaluation. Presence of periodontitis if PSR 3 or 4.	Cross –Sectional	P: 5.5 (5.3–5.8) DMP: 6.6 (6.0–7.8) (median and interquartile range)	Saliva, Serum; IL-17 A, IL-18	*A trend of higher IL-17 levels in individuals with diabetes with periodontitis; in the correlation with RSP, salivary levels were increased in patients with RSP4 compared with the RSP0 and RSP1 group; salivary and serum levels of IL-18 did not show statistically significant differences; individuals with high glycated hemoglobin levels tended to show higher serum levels of IL-18; no correlation between serum and salivary levels was observed.

Table 2. Summary of the results of the qualitative, quantitative and certainty analyses of the evidence produced for the inflammatory mediators tested in the studies included.

Inflammatory markers	Studies included	Group P (n)	Group DMP (n)	Analysis	Studies/findings	Meta-analysis	Certainty of evidence (grade)
IL-10	[19, 21, 28, 29, 31]	109	119	Serum	**19,28,31:** Group P minor expression (p > 0.05)	19,28,31	⊕OOO
					21: Group P minor expression (p = NA)	md: -0.88	very low
					29: Group P highest expression (p < 0.05)	favored group P (p < 0.05)	
IL-8	[26, 28]	32	50	Serum	**26:** No numerical data available (p > 0.05)		
					28: Group DMP highest expression (p > 0.05)		
IL-6	[20, 21,24, 26, 28]	87	105	Serum	**20,24:** Group DMP highest expression (p < 0.05)	**24,28**	⊕OOO
					21: Group DMP highest expression (p = NA)	md: -8.45	very low
					26: No numerical data available (p > 0.05)	favored group P (p > 0.05)	
					28: Group P highest expression (p > 0.05)		
					22: Group DMP highest expression (p =NA)		
					27: Group DMP highest expression (p > 0.05)		
	[22, 27]	44	44	Saliva			
IL-4	[20, 21, 24, 28, 29]	113	117	Serum /Plasma	**20,24,28:** Group P highest expression (p > 0.05)	**24,28**	⊕OOO
					21: Group P highest expression (p = NA)	md: 5.45	very low
					29: Group P highest expression (p < 0.05)	favored group DMP (p < 0.05)	
IL-2	[28]	26	30	Serum	Group P highest expression (p < 0.05)		
TNF-□	[20, 21, 24, 28, 31]	97	107	Serum /Plasma	**20,24** Group DMP highest expression (p < 0.05)	**24,28**	⊕OOO
					21: Group DMP highest expression (p = NA)	md: -5.62	very low
					28,31: Group P highest expression (p > 0.05)	favored group P (p > 0.05)	
					02: Group DMP highest expression (p < 0.05)		
	[2]	20	20	Saliva			
IL-1□	[21, 28]	46	50	Serum	**21:** Group DMP highest expression (p = NA)		-
					28: Group P highest expression (p > 0.05)		
IL-34	[23]	35	35	Plasma	Group DMP highest expression (p > 0.05)		-
IL-23	[28, 30]	44	48	Serum	**28,30** Group P highest expression (p > 0.05)		-

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IL-35	[30]	18	18	Serum	Group P highest expression (p > 0.05)	-
IL-17	[28, 32]	51	87	Serum	Serum: **28:** Group P highest expression (p > 0.05) **32:** No numerical data available (p > 0.05) **32:** No numerical data available (p >0.05). **25:** Group DMP highest expression (p = NA)	-
	[25,32]			Saliva		
IL-18	[32]	8	23	Serum /Saliva	Serum: **32:** No numerical data available (p > 0.05) saliva: **32:** No numerical data available (p > 0.05)	-
ADIPOCYTOKINE	[31]	16	22	Serum	Group P highest expression (p < 0.05)	-
IL-5, IL-7, IL-12, IL-13, IL-21; TGF-B; E IFN-Γ	[28]	26	30	Serum	**IL-12 E IFN-Γ:** Group P highest expression (p < 0.05) **IL-7:** Group P highest expression (p > 0.05) **IL-5,IL-13,IL-21, TGF-B:** Group DMP highest expression (p > 0.05)	-

NA: not available.

Methodological quality assessment

The methodological quality of the studies included was assessed by Joanna Briggs Critical Appraisal Tools.[16] Each study design (cohort, case-control, or cross-sectional) evaluated by this tool has its own checklist, and the answers provided to analyze the criteria may be "yes" (when fully meeting the criteria), "no" (when not meeting the criteria), "not clear" or "not applicable." Two independent reviewers performed the analysis. Conflicts were solved by consensus. In the case of disagreement, a consensus was reached at a meeting with the other researchers of the study. The studies were evaluated as "low quality" when a total of 49% of "yes" answers were reached; "moderate quality" when "yes" answers were between 50-69%; and "high quality" when attaining 70% or more of "yes" answers. The risk of bias assessments can be accessed at https://osf.io/a53bc/files/.

Outcomes

The main outcomes were the concentrations of pro- and anti-inflammatory markers in the blood and saliva samples of individuals with periodontitis, whether with or without T2D (comparison arm). The studies that did not present quantitative results for the markers were analyzed qualitatively.

Meta-analysis

The effect measure adopted was the mean difference, considering the concentration of the biomarkers as a continuous variable. The heterogeneity assessment was performed considering the direction and size of the effect estimates in the forest plot, the results of the chi-squared test, and the I^2 scores. When all the indicators suggested heterogeneity, the studies were considered heterogeneous.[17] Studies that presented results in pg/ml were selected for the meta-analysis. The study by Bakshi et al.[24] did not mention the unit of measurement; however, the methodology inferred that the unit was expressed in pg/ml, because of how the values were quantified. An email was sent to the authors to obtain confirmation, but no response was obtained. The statistical tool adopted was the inverse variance method using a random-effects approach. The meta-analysis was performed using the Review Manager 5.3 software

program (Nordic Cochrane Center, Copenhagen, Denmark). The GRADE (Grading of Recommendations Assessment, Development and Evaluation) system[18] was used to consolidate the certainty of the evidence gathered. The GRADE can be accessed at https://osf.io/a53bc/files/.

Results

Location of the studies

The studies included were conducted in India,[2,19-25] Brazil,[26-28] Ukraine,[29] Iran,[30] Indonesia,[31] and Thailand.[32] All the articles were published in English, except the Ukrainian study, which was translated into English using the DeepL online translator (*Köln*, Germany) before the analysis was performed.

Data analysis and presentation

The database search yielded a total of 3,894 studies. The duplicates were removed (n = 1,840), leaving 2,054 titles and abstracts to be evaluated. Of this total, 22 articles remained for the phase two full text reading, which led to the exclusion of 7 studies (the list of the excluded articles with reasons for exclusion can be accessed at https://osf.io/a53bc/files/). This left 15 studies that met the inclusion criteria for performing the qualitative analysis, 4 of which were included in the quantitative analysis. The manual search of the reference lists of included studies did not recover any new studies, nor did the consultation with the experts. The flowchart of the studies selection is shown in Figure 1.

The 15 cross-sectional papers that were selected represented a total of 292 patients in the periodontitis group, and 348 patients in the T2D group with periodontitis. The authors were contacted to ascertain if the same population was used in the three studies by Acharya et al.[19; 20; 21] but did not respond. Because no mention was made in this regard, the populations were considered as being different, but were not included in the same meta-analysis. The mean age of the participants of the studies ranged between 30 and 65 years old. According to the methodological quality analysis, none of the articles met all of the

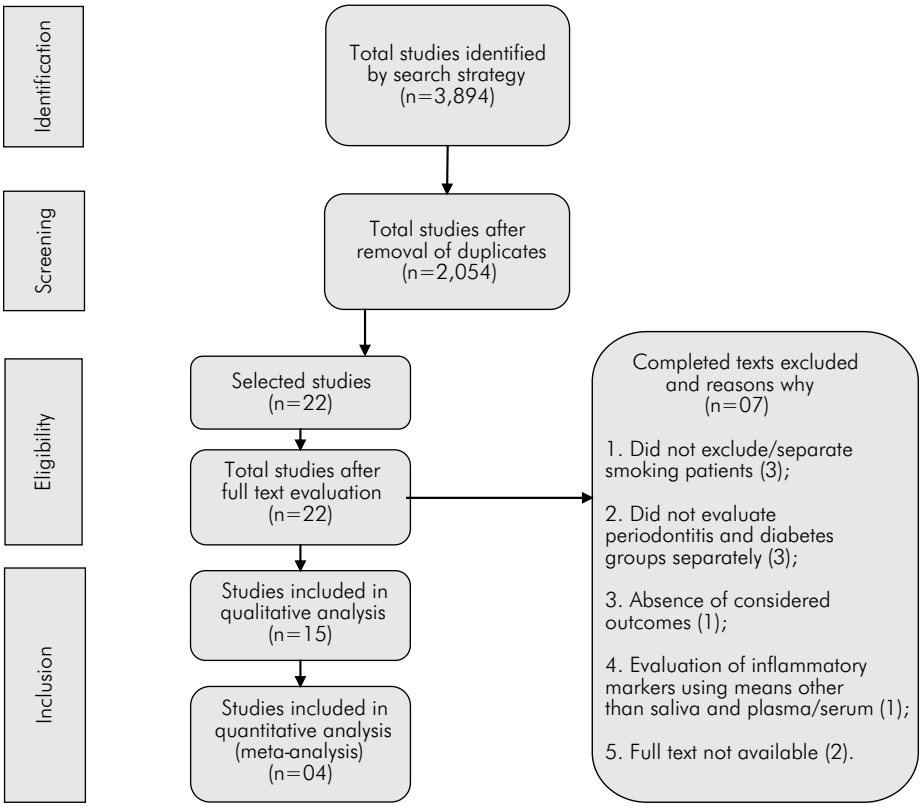

Figure 1. Flow diagram of the search and selection process of the studies (adapted from PRISMA).

criteria specified. The confounding factors and the strategy for dealing with these factors were not clearly set out in the description of the paper. Three articles did not objectively describe the inclusion and exclusion criteria,[21,22,29] and two studies did not define the criteria with clarity.[24;30] The characterization of the population and the scenario were not clear in one article,[30] and were not presented in three others.[21,22,29] The parameters used in relation to the objectivity of the criteria were not clearly defined in three articles.[21,22,29] The appropriate statistical analysis was not detailed in four studies.[21,22,29,31] Among the studies evaluated, nine were classified as high quality,[2,19,20,23,25-28,32] four, as low quality,[21,22,24,29] and two, as medium quality.[30,31] The risk of bias assessments can be accessed at https://osf.io/a53bc/files/.

Individual results

Studies that presented no quantitative results for the biomarker levels, or results expressed by mean and standard deviation were not considered for the quantitative analysis. Therefore, the meta-analysis could only be performed for the levels of IL-10, IL-6, IL-4, and TNF-α. Four studies were included in the meta-analysis.[19,24,28,31]

Figure 2 shows that the studies by Miranda et al.,[28] and by Purnamasari et al.[31] presented no significant statistical differences in the IL-10 levels, between the periodontitis and the diabetes with periodontitis groups. However, Acharya et al.[19] found a significant difference between the two groups. The meta-analysis showed that the level of IL-10 was significantly lower in the periodontitis group than in the T2D plus periodontitis group (p = 0.003).

The studies involving IL-6 are shown in Figure 3. Only two studies could be synthetized quantitatively.[24,28] The inconsistency rate was 100%, indicating heterogeneity between the studies. The results of Bakshi et al.[24] had a greater weight in the analysis, with lower levels of IL-6 for the periodontitis group. In contrast, the study by Miranda et al.[28] showed no significant statistical difference between the two groups. The final evaluation of this systematic review pointed out that there was no statistical difference between the groups evaluated.

In the TNF-α analysis, as shown in Figure 4, two studies provided data for inclusion in the meta-analysis.[24,28] Both presented heterogeneity, and no significant statistical differences were found in their final evaluation. The evaluation of the IL-4 studies (Figure 5) pointed out a 0% inconsistency rate. Moreover, the meta-analysis that includes the two studies[24, 28] showed a significantly lower mean IL-4 concentration in the diabetes periodontitis group than in the non-diabetic periodontitis group (p < 0.001).

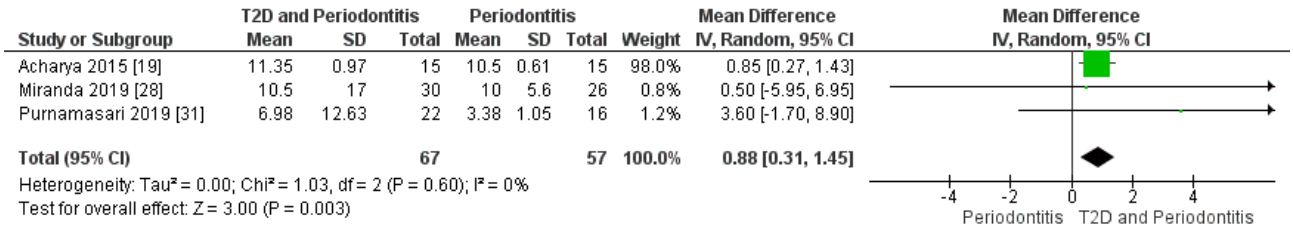

Figure 2. Forest plot for IL-10 levels in the plasma of the periodontitis and the diabetes plus periodontitis groups.

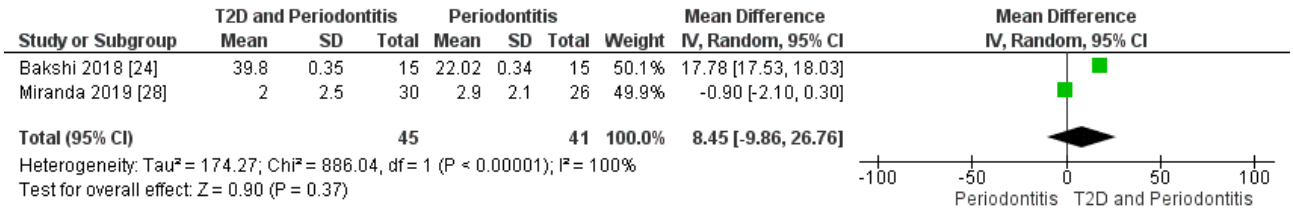

Figure 3. Forest plot for IL-6 levels in the plasma of the periodontitis and the diabetes plus periodontitis groups.

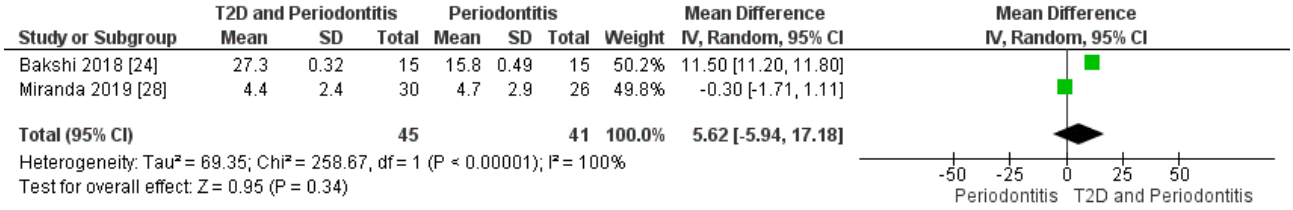

Figure 4. Forest plot for TNF- α levels in the plasma of the periodontitis and the diabetes plus periodontitis groups.

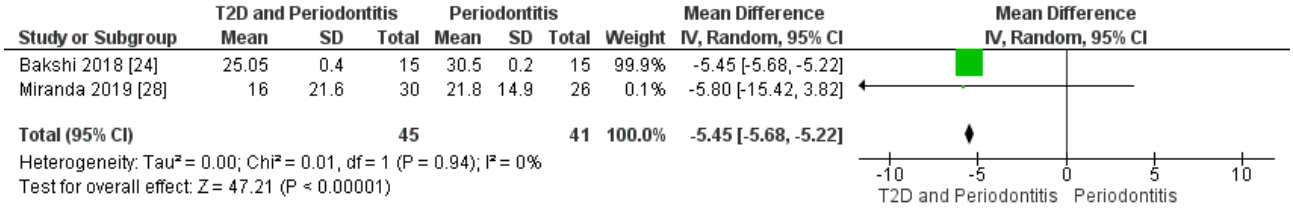

Figure 5. Forest plot for the IL-4 plasma levels of the periodontitis and the diabetes plus periodontitis groups.

Discussion

This study evaluated the expression of anti- and pro-inflammatory mediators in the saliva and plasma of T2D patients with periodontitis. A table with studies at p-value (Table 2) was inserted because the data on mean differences and CI could not be presented for all the biomarkers considered 33. The most expressive results were found for the levels of IL-10 and IL-4 expression, which are directly related to the periodontitis and diabetes mellitus processes. The increased concentration of the anti-inflammatory interleukin-10 (IL-10) in patients with periodontitis and diabetes may account for the impairment of the immune response of these individuals. This hypothesis may explain the severity of the infectious inflammatory process, in the course of both periodontitis and T2D.

In periodontitis, IL-10 levels can deregulate the synthesis of pro-inflammatory cytokines.[34] IL-10 is an anti-inflammatory mediator, and the main regulator of inflammatory responses; it plays an important role in the pathogenesis of periodontitis.[35] IL-10 levels in patients with periodontitis tend to be lower in comparison with those of healthy patients, and this decrease may cause greater susceptibility of the individual in developing chronic periodontitis.[35] In this systematic review, the results of the meta-analysis (Figure 2) indicated a significantly higher serum level of IL-10 in T2D patients with periodontitis than in just periodontitis patients. This suggests that IL-10 may play a relevant role in modulating the immune response in T2D patients with periodontitis. The fact that patients with periodontitis have lower levels of IL-10 may explain the change in the immune response. The exacerbation of this cytokine in T2D patients with periodontitis may reveal a modulation of this immune system to combat both inflammatory diseases.

IL-8 is a mediator secreted by several cell types during inflammatory stimulation, and is associated with the initiation and amplification of the acute inflammatory reaction process.[36] IL-8 levels correlate with diabetes associated to periodontal disease, and this association can underscore the importance of this marker in the pathogenesis of both diabetes and periodontitis.[36] However, in the qualitative evaluation of this systematic review, the results of IL-8 expression showed no statistically significant differences between the periodontitis plus diabetes and the just periodontitis groups.

IL-6 is a pro-inflammatory mediator directly related to diabetes, since it induces insulin resistance and periodontal disease in the process of bone resorption.[37] This marker is synthesized during infectious stimulation or aggression and activates the acute immune response.[37] The salivary levels of IL-6 tend to reflect blood levels.[38] The qualitative analyses of serum and saliva performed in this

systematic review showed that the levels of this mediator were higher in patients with both diabetes and periodontitis.[20-22,24,27] However, the quantitative analyses did not show a statistically significant difference between the periodontitis plus T2D and the just periodontitis groups.

Like IL-10, IL-4 is an anti-inflammatory mediator related to the pathogenesis of periodontitis.[39] The analysis of this marker in the studies included in this systematic review[20,21,24,28,29] demonstrated high levels of IL-4 in the periodontitis groups. Our meta-analysis revealed a lower expression of IL-4 in T2D patients with periodontitis than in non-diabetic patients with periodontitis. This could explain the important involvement of this cytokine in periodontitis pathogenesis, periodontitis progression in diabetic patients, and the higher susceptibility of these patients to periodontitis. One hypothesis explaining the higher level of IL-4 – an anti-inflammatory cytokine – in patients with periodontitis versus those with both diseases, is that IL-4 may suffer the indirect or direct influence of some other inflammatory mediator present in T2D patients with periodontitis. The decrease in IL-4 in diabetic patients with periodontitis may reflect a change in the immune response pattern, since these patients are more susceptible to inflammation.

Another immunoregulatory, pro- and anti-inflammatory cytokine that impacts bone resorption is IL-2.[40] The results of the studies presented in this systematic review show a high expression of IL-2 in the periodontitis group,[28] thus highlighting the role of this marker in periodontal disease. IL-2 is largely associated with the chronic inflammatory process of Type 1 diabetes, caused by a change in the signaling cascade.[41] However, there are not many reports of such processes in association with T2D.

TNF-α is a pro-inflammatory mediator directly related to periodontitis and T2D. This marker induces insulin resistance, and also leads to chronic systemic inflammation.[42] A pattern of high expression of TNF-α in the periodontitis groups with diabetes could be seen in the qualitative analyses.[2,19,20,23] On the other hand, the meta-analysis, together with the two selected studies, presented no significantly statistical differences in the TNF-α serum concentrations in the two groups of patients. This result may have been affected by the size and heterogeneity of the samples. This systematic review and meta-analysis showed that the literature concerning the concentration of cytokines in the saliva of T2D individuals is very scarce, as could be anticipated by our own failed attempt to perform a quantitative analysis in saliva. Future studies should focus on gaining a better understanding of the salivary levels of the inflammatory cytokines in T2D.

IL-12 and IFN-γ were evaluated qualitatively. The selected studies showed high levels of IL-12 and IFN-γ expression in the periodontitis versus the diabetes with periodontitis groups, with a statistically significant difference among the groups.[27] The first marker is pro-inflammatory, which tends to be increased in periodontitis patients, compared with healthy patients, thus indicating a pro-inflammatory response.[43] The second marker is an effector cytokine with immunomodulatory properties that coordinate the immune response.[44] Further studies are required to explain and correlate the levels of these markers in periodontitis in association with diabetes mellitus.

Improvement

An understanding of the inflammatory cytokine expression affords an overview of the immune system, and can help characterize immune response patterns in different individuals. This knowledge may provide valuable information regarding the susceptibility to periodontal disease and/or diabetes mellitus. Several inflammatory mediators are involved in the process of progression or attenuation of inflammation during the immune response, and patients tend to have higher or lower rates of tissue destruction, depending on the production of cytokines in the inflammatory process. A salivary analysis would be considered very practical for evaluating these mediators in periodontal disease, considering the low cost and easy procedure of saliva collection, unlike the invasive clot-risking process of collecting blood samples. However, the results presented by the selected studies that investigated cytokines in the saliva in this systematic review did not detect any significant differences in the comparisons between the groups. This lack of quantitative results meant that the studies could not be included in the meta-analysis. Moreover, although salivary samples are

practical to collect, the collection process must be well executed. For instance, should blood be collected with the salivary samples, this will affect the analysis of the results [38]. This points out the challenge faced by the authors of the studies that were included, in regard to analyzing the saliva samples.

Evaluations of inflammatory markers in saliva may suggest the progression of periodontal disease in patients with Type 2 diabetes. This detection enables an individualized and resolutive treatment plan. The less complex mechanism of saliva collection makes laboratory analysis feasible for clinicians. However, studies looking at the behavior of systemic mediators at local levels are still needed. The impossibility of including studies with salivary samples in the meta-analysis demonstrates the scarcity of studies. Therefore, understanding the performance of these mediators at salivary levels, with their consequent oral impact, becomes more complex.

Conclusion

Review update

Considering that our research aimed to provide information on inflammatory mediators in periodontitis whether or not associated with T2D, it can be seen that evidence still lacks on these markers. There is a gap in the literature regarding answers to questions on normal or altered levels of inflammatory cytokines in patients with periodontitis and T2D. The evaluations of these mediators were performed by several studies; however, a difference was noticed in the presentation of the results, regarding the quantification or different measurement of the data.

Caution is warranted in inferring the results, since the meta-analysis did not include all the cytokines. However, the results presented in both quantitative and qualitative analyses, mostly corroborate each other. Despite the promising results, it is fundamental to standardize the presentation of results and the protocols, so that the results can be more feasible, and hence better for drawing comparisons. Additional data are needed to investigate the expression of these biomarkers in the saliva and serum of patients with periodontitis and T2D, thus enabling better results and conclusions.

It can be concluded that the IL-4 expression was lower in T2D patients with periodontitis than patients with periodontitis. This result suggests a possible change in the immune response pattern in T2D patients. The increase in IL-4 expression in patients with periodontitis versus patients with both diseases suggests the influence of other inflammatory response pathways on the expression of this cytokine in the presence of periodontitis and diabetes occurring together. The quantitative analysis was limited; hence, no definitive conclusion could be reached regarding other markers.

It is important to emphasize that the studies included in the meta-analysis presented heterogeneity, and the low number of these studies directly influenced the results obtained. Thus, it is suggested that further studies make another evaluation of the expression of these inflammatory mediators in patients with periodontitis and Type 2 diabetes.

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